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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Pyrimethanil

FROM: Yung G. Yang, Ph.D. *Yung G. Yang 1/28/97*
Review Section II
Toxicology Branch II
Health Effects Division (7509C)

and

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Manager, Carcinogenicity Peer Review Committee
Science Analysis Branch
Health Effects Division (7509C)

THROUGH: Stephanie R. Irene, Ph.D. *Stephanie R. Irene*
Deputy Director, Health Effects Division (7509C) *2/11/97*

TO: Connie Welch
Product Manager #21
Fungicide-Herbicide Branch
Registration Division (7505C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on May 29, 1996 to discuss and evaluate the weight-of-the-evidence on pyrimethanil with particular reference to its carcinogenic potential. The CPRC concluded that pyrimethanil should be classified as Group C - possible human carcinogen - and recommended that a Margin of Exposure (MOE) methodology be used for the estimation of human risk. The MOE methodology was selected because the thyroid tumors associated with administration of pyrimethanil in Sprague-Dawley rats may be due to a disruption in the thyroid-pituitary status.

1/37

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SUMMARY

Administration of pyrimethanil in the diet to CD-1 mice at doses up to 1600 ppm did not result in increases in any tumors; however, the highest dose was not considered to have been adequate for assessing the carcinogenic potential of pyrimethanil in the mouse.

Administration of pyrimethanil in the diet to Sprague-Dawley rats resulted in an increase in thyroid follicular cell tumors in both sexes. In male rats there was a statistically significant increase in thyroid follicular cell combined adenoma/carcinoma at the highest dose (5000 ppm) and a borderline statistically significant increase in adenomas ($p=0.0505$). There were also statistically significant positive dose-related trends for thyroid follicular cell adenomas and combined adenoma/carcinoma in male rats. In female rats there was a statistically significant increase in adenomas at the highest dose (5000 ppm) with a statistically significant positive dose-related trend. The tumor incidences in both sexes exceeded the upper range of historical controls. The adenomas in male rats occurred with an early onset, based on the findings at the interim 1 year sacrifice.

The CPRC considered the highest dose in female rats to have been adequate for assessing the carcinogenic potential of pyrimethanil; however, dosing in male rats could have been higher.

Pyrimethanil is a new chemical for which no suitable analogs could be found and it does not appear to have mutagenic activity.

The classification of Group C was based on the increases in thyroid follicular cell tumors in both sexes of the Sprague-Dawley rat, statistically significant by both pair-wise and trend analysis. The MOE approach was selected because there appeared to be sufficient evidence for relating the thyroid tumors in the rat to a disruption of the thyroid-pituitary status (a full discussion of this analysis is found in the body of the document - Section F, number 6). Therefore, a threshold consideration (M.O.E.) was to be applied in estimating risk.

A. Individuals in Attendance at the meetings:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Stephanie Irene

Karl Baetcke

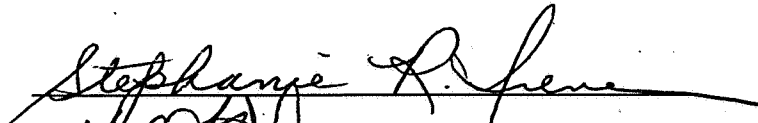
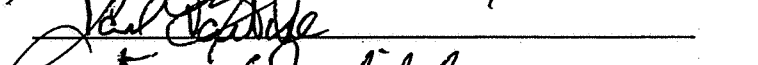

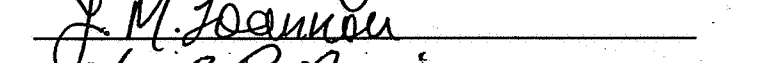

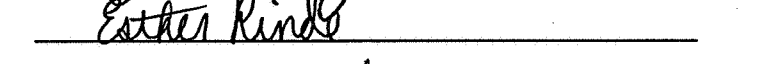
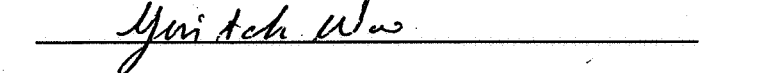
Kerry Dearfield

Yiannakis Ioannou

Hugh Pettigrew

Esther Rinde

Yin Tak Woo

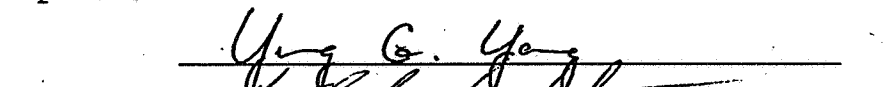
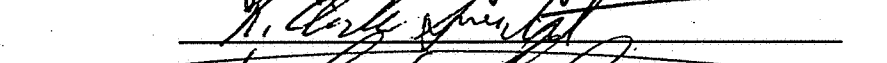
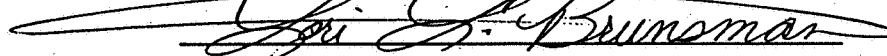








2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Yung Yang¹

Clark Swentzel

Lori Brunsman

3. Other Attendees: Bernice Fisher, Linda Taylor, Deborah McCall (HED)

¹Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

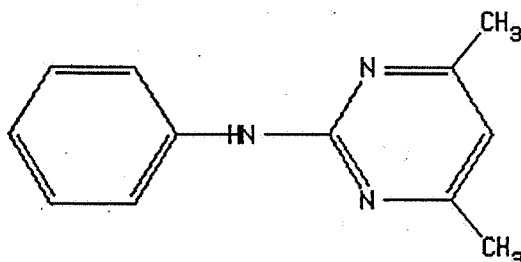
B. Material Reviewed

The material available for review consisted of DER's, one-liners, data from the literature and other data summaries prepared and/or supplied by Dr. Yang, and tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report.

C. Background Information

Pyrimethanil [4,6-dimethyl-N-phenyl-2-pyrimidinamine] is a foliar fungicide for the control of grey mold (*Botrytis cinerea*) on grapes, strawberries, tomatoes, and ornamentals. Pyrimethanil is registered, at present, only in France for use on grapes and strawberries under the trade name of SCALA®. The CAS Registry Number (CAS No.) is 53112-28-0. The PC Code is 288201.

The chemical structure is:



Pyrimethanil

D. Evaluation of Carcinogenicity Evidence

1. Carcinogenicity Study in Mice

Reference: Clay, H.: Technical SN 100 309: 80 week oral (Dietary Administration) Carcinogenicity Study in the Mouse. March, 1992. MRID# 43301615, Doc.# 011695. Study #: HUK# 194/42. Test facility: Schering Agrochemical Ltd. England.

a. Experimental Design

Groups of Cr1:CD*®-1(ICR)BR mice [51/sex/dose] were administered pyrimethanil via diets at dose levels of 0, 16 ppm [♂ 2.0/♀ 2.5 mg/kg/day], 160 ppm [♂ 20.0/♀ 24.9 mg/kg/day], or 1600 ppm [♂ 210.9/♀ 253.8 mg/kg/day] for 80 weeks.

b. Discussion of Tumor Data

MALES: The incidence of neoplastic lesions observed was comparable among the groups. One male at each dose level displayed a Leydig cell tumor, but none was observed in the control group. Two high-dose males displayed benign adenomas in the adrenal gland.

FEMALES: The incidence of neoplastic lesions observed was comparable among all groups.

c. Non-Neoplastic Lesions

MALES: UROGENITAL TRACT: The incidence of urogenital tract lesions was increased in treated males during the first 52 weeks with the highest incidence being observed at the high-dose level. These lesions were characterized by balanoposthitis of the penis, preputial gland adenitis/abscess, extension/vesiculitis in the seminal vesicles, coagulating gland distension and prostatitis and urinary bladder distension/cystitis. The significance of the increase in these lesions is not clear, but these are consistent with the findings at necropsy. **Eye:** Increased incidences of lenticular degeneration were noted at the high dose compared to the control. **SPLEEN:** Extramedullary erythropoiesis was increased slightly at the high dose compared to the control [Table 1].

FEMALES: SCIATIC NERVE: Neuropathy was increased slightly in the high-dose females compared to the controls. **LIVER:** Several lesions were slightly increased at the high-dose level, but all are consistent with the normal findings in older mice. **KIDNEY:** Several kidney lesions were increased slightly at the high dose compared to the control group, but all are consistent with the normal findings in older mice. **PANCREAS:** The incidence of Islet cell hyperplasia was more than double that found in the control group, but all of the low- and mid-dose pancreases were not examined, so an assessment of a dose response is not possible. **SPLEEN:** Extramedullary erythropoiesis was increased slightly at the high dose compared to the control [Table 2].

Tissue/Lesion/ Dose (ppm) MALES	Table 1. Microscopic Findings - Non-Neoplastic			
	0	16	160	1600
♂ Urinary Bladder N= distension cystitis urothelial hyperplasia	51 4 1 1	27 7 6 1	17 4 3 0	51 9 5 3
♂ Prostate N= distension coagulating gland adenitis prostatitis coagulating gland distension	50 0 0 2 21	31 0 2 3 16	19 0 1 4 10	51 1 4 6 20
♂ Penis N= urethral plug balanoposthitis	2 0 1	7 0 7	2 0 1	10 1 9
♂ Preputial Gland N= adenitis/abscess	12 4[33]♦	15 8[53]	13 9[69]	18 14[78]
♂ Ureter N= distension	0 0	1 1	1 1	1 1
♂ Urethra N= urethritis	0 0	0 0	0 0	1 1
♂ Epididymis N= oligospermia	51 9[18]	29 4[14]	16 1[6]	51 12[24]
♂ Seminal Vesicle N= distension vesiculitis	51 18[35] 3[6]	31 12[39] 1[3]	20 9[45] 5[25]	51 25[49] 5[10]
♂ Eyes N= lenticular degeneration	49 3[6]	27 1[4]	16 1[6]	48 7[15]
♂ Spleen N= extramedullary erythropoiesis	51 17[33]	28 16[57]	17 5[29]	51 25[49]
♂ Thyroid N= focal follicular distension	51 31[61]	28 17[61]	16 14[88]	51 29[57]

♦ [%]

Tissue/Lesion/ Dose (ppm) FEMALES	Table 2. Microscopic Findings - Non-Neoplastic			
	0	16	160	1600
♀♀ Sciatic Nerve N= neuropathy	51 29[57]♦	11 3[27]	12 5[42]	51 36[71]
♀♀ Pancreas N= islet cell hyperplasia	51 6[12]	11 2[18]	12 0	51 13[25]
♀♀ Ovaries N= luteal hyperplasia	51 1[2]	29 0	34 0	51 3[6]
♀♀ Liver N= hepatocyte vacuolation Kupffer cell pigment focal necrosis extramedullary erythropoiesis	51 20[39] 9[18] 9[18] 1[2]	51 21[41] 4[8] 6[12] 1[2]	51 22[43] 4[8] 7[14] 1[2]	51 26[51] 13[25] 10[20] 3[6]
♀♀ Kidney N= glomerulonephropathy tubular atrophy/cystic renal corpuscles cysts inflammatory cell foci	51 30[59] 7[14] 11[22] 45[88]	51 37[73] 9[18] 14[27] 47[92]	51 31[61] 13[25] 9[18] 47[92]	51 35[69] 11[22] 16[31] 49[96]
♀♀ Spleen N= extramedullary erythropoiesis	51 15[29]	19 8[42]	17 7[41]	51 22[43]
♀♀ Thyroid N= focal follicular distension	51 25[49]	11 3[27]	12 5[42]	51 29[57]

♦ (%)

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

Although there were increases in the number of deaths in the treated males relative to the control, there were adequate numbers of mice at each dose level at termination for an assessment of the carcinogenic potential. However, the CPRC did not consider the highest dose to have been adequate for assessing the carcinogenic potential of pyrimethanil in these mice.

The author stated that 10000 ppm [♂♂ 1864/♀♀ 2517 mg/kg/ day] fed for 90 days [Study # TOX 90212] resulted in urinary bladder stones composed of compound-related material, and the size of the stones at 90 days was such that 10000 ppm was considered too high for a lifetime exposure period. TB II notes that the incidence of stones was low in males [1/10] and moderate [4/10] in females in the 90-day study, and the other urinary bladder lesions [uroliths in lumen, urothelial hyperplasia] also occurred at the 10000 ppm dose only. The next highest dose level used in the 90-day study was 900 ppm, a dose at which no lesions were observed. The Registrant's arguments for/ justification of the dose levels tested do not support the selection of 1600 ppm for the high dose in the current study, in light of the results of the 28-day study and the selection of inadequate mid- and low-dose levels for the 90-day study. Since the conclusion of the 28-day study was

that a dose between 3000 ppm and 10000 ppm would be suitable as the high-dose for the 90-day study and 10000 ppm was chosen, it is not apparent why the mid-dose level for the 90-day study was not 3000 ppm or greater or one-half the high dose, for example. The NOEL for systemic effects can be set at 1600 ppm [$\delta\delta$ 210.9/ ♀♀ 253.8 mg/kg/day], the highest dose tested [HDT]. There was no increase in the incidence of any tumor type in either sex.

2. Combined Chronic Toxicity/Carcinogenicity Study in Rats

Reference: Bright, JHM: (T25/2A) Determination of SN 100 309 Dietary Concentrations for a 104 week Rat Combined Chronic Toxicity and Oncogenicity Study. November 2, 1993. MRID# 43301612 & 43301613; Doc. # 011695. Study #: TOX/90437; Report #: RESID/92/88. Test facility: Schering Agrochemical Ltd. England.

a. Experimental Design

Sprague-Dawley rats (50/sex/group for 104 weeks; 20/sex/group for interim 52-week sacrifice) were fed pyrimethanil (SN 100 309) at dose levels of 0, 32 ppm (δ /1.3 ♀ /1.8 mg/kg/day), 400 ppm (δ /17 ♀ /22 mg/kg/day), or 5000 ppm (δ /221 ♀ /291 mg/kg/day).

b. Discussion of Tumor Data

Overall, the only tissue showing a higher incidence of tumors than controls was the thyroid gland with benign follicular cell adenomas in 5000 ppm treated animals (9/70 males and 7/70 females) compared to control (3/70 males and 0/70 females) (Table 3). In females, the incidence at the high dose (7/70 or 10%) was higher than the control (0/70) and the historical control range (0% - 3.0%). In addition, thyroid follicular cell adenocarcinomas were seen in animals treated at 32 ppm (1/70 male) or at 5000 ppm (1/70 male only); however, the incidence (1.4%) was within the historical control range. The historical control data were attached.

There was no significant finding at 400 ppm or 32 ppm.

Table 3. Incidence of Neoplastic Findings in the Thyroid Gland					
Male					
	Dose Level (ppm)				Historical Control [§] % (range)
	0	32	400	5000	
Follicular cell adenoma	3/70 (4.3%)	3/70 (4.3%)	2/70 (2.9%)	9/70 (12.9%)	3.9% (1.5-5.9)
Follicular cell adenocarcinoma	0/70	1/70 (1.4%)	0/70	1/70 (1.4%)	1.2% (0-2.9)
Females					
Follicular cell adenoma	0/70	3/70 (4.3%)	3/70 (4.3%)	7/70 (10%)	1.1% (0-3.0)
Follicular cell adenocarcinoma	0/70	0/70	0/70	0/70	0.3% (0-1.5)

[§] Historical control data from AgrEvo USA Company, 1995.

The historical control data are collected from 5 groups of males and female CD rats.

The following statistical evaluations on survival and tumor analyses are provided by Science Analysis Branch (memo from L Brunsman to Y Yang, May 1, 1996).

Survival Analyses

The statistical evaluation of mortality indicated significant decreasing trends for mortality with increasing doses of pyrimethanil in male and female rats.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analyses

Male rats had significant increasing trends for thyroid follicular cell adenomas and adenomas and/or carcinomas combined, both at $p < 0.01$. There was a significant difference in the pair-wise comparison of the 5000 ppm dose group with the controls for thyroid follicular cell adenomas and/or carcinomas combined at $p < 0.05$. There was also a significant increasing trend for thyroid follicular cell adenomas in the interim sacrifice group at $p < 0.05$.

Female rats had a significant increasing trend, and significant differences in the pair-wise comparisons of the 32 and 5000 ppm dose groups with the controls, for thyroid follicular cell adenomas, all at $p < 0.05$.

The statistical analysis of the male and female rats was based upon Peto's Prevalence Test since there were statistically significant negative trends for mortality with increasing doses of pyrimethanil in male and female rats. Interim sacrifice animals have been included in the Peto analyses because the tumors that appeared at the interim sacrifice occurred at the high dose only and were statistically significant in the males. See Tables 4, 5 and 6 for tumor analysis results.

Table 4. Pyrimethanil - Sprague-Dawley Crl:CD(SD)BR Rat Study

Male Thyroid Follicular Cell Tumor Rates*
and Peto's Prevalence Test Results (p values)

	<u>Dose (ppm)</u>			
	0	32	400	5000
Adenomas (%)	3/65 (5)	3/68 (4)	2/65 (3)	9 ^a /68 (13)
p =	0.004**	-	-	0.0505
Adeno- carcinomas (%)	0/19 (0)	1 ^b /24 (4)	0/23 (0)	1/28 (4)
p =	0.278	0.187	-	0.205
Combined (%)	3/65 (5)	4/68 (6)	2/65 (3)	10/68 (15)
p =	0.003**	0.500	-	0.034*

*Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

^aFirst adenoma observed at week 54, dose 5000 ppm, in an interim sacrifice animal.

^bFirst adenocarcinoma observed at week 107, dose 32 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Pyrimethanil - Sprague-Dawley Crl:CD(SD)BR Rat Study

Female Thyroid Follicular Cell Tumor Rates*
and Peto's Prevalence Test Results (p values)

	<u>Dose (ppm)</u>			
	0	32	400	5000
Adenomas* (%)	0/67 (0)	3/68 (4)	3/67 (4)	7/68 (10)
p =	0.028*	0.041*	0.080	0.014*

*Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

^aFirst adenoma observed at week 53; dose 5000 ppm, in an interim sacrifice animal.

*No adenocarcinomas were observed.

Note:Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Pyrimethanil - Sprague-Dawley Crl:CD(SD)BR Rat Study
Thyroid Follicular Cell Interim Sacrifice Adenoma Rates* and
Exact Trend Test and Fisher's Exact Test Results (p values)

	<u>Dose (ppm)</u>			
	0	32	400	5000
Males	0/19	0/19	0/20	3/20
(%)	(0)	(0)	(0)	(15)
p =	0.015*	1.000	1.000	0.125
Females	0/19	0/19	0/19	1/20
(%)	(0)	(0)	(0)	(5)
p =	0.260	1.000	1.000	0.513

*Number of tumor bearing animals/Number of animals examined at week 53.

Note:Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

c. Non-neoplastic

Interim Kill (52 weeks) (Table 7 and 8)

At 5000 ppm, minimal to moderate hypertrophy of centrilobular hepatocytes were observed in all males and 1/20 females but was not seen in the controls of either sex. In the thyroid gland, there were minimal to moderate intra-epithelial depositions of brown pigment in 18/20 males and 19/20 females compared to 1/19 of controls of both sexes. There were higher incidences of colloid depletion and hypertrophy of the follicular epithelium (18/20 in males and 13/20 in females for each) than controls (9/19 males and 6/19 females for each).

No significant treatment-related effect was reported at 400 or 32 ppm.

Terminal Kill (104 weeks) (Table 7 and 8)

Minimal to slight hypertrophy of centrilobular hepatocytes was observed in animals given 5000 ppm only (32/50 males and 6/50 females). Also, there were higher incidences of minimal to severe eosinophilic foci (19/50 males and 12/50 females) than in controls (2/51 males and 7/51 females). In addition, minimal to moderate focal cystic degeneration was observed in males (30/50, 28/50, 17/51, or 15/51 at dose levels of 5000, 400, 32 ppm, or controls, respectively) while there was no apparent effect in females.

In the thyroid gland, minimal to severe colloid depletion and hypertrophy of follicular epithelium were observed at 5000 ppm in both sexes at higher incidence (36/50 males and 38/50 females) than controls (25/51 males and 15/51 females). Focal hyperplasia of the follicular epithelium was seen at 5000 ppm in 9/50 males and 7/50 females compared with controls (2/51 males and 1/51 females). Minimal to moderate depositions of intra-cytoplasmic brown pigment (lipofuscin) in the thyroid follicular epithelium were detected at 5000 ppm in both sexes (38/50 males and 47/50 females) but was not present in controls of either sex.

Table 7. Incidence of Significant Microscopic Findings in Males								
Parameter	Dose Level (ppm)							
	0		32		400		5000	
	I	T	I	T	I	T	I	T
No. of animals	19	51	19	51	20	50	20	50
<u>Liver</u>								
Hypertrophy of centrilobular								
minimal	0	0	0	0	0	0	7	6
-slight	0	0	0	0	0	0	12	26
-moderate	0	0	0	0	0	0	1	0
Total	0	0	0	0	0	0	20	32
Eosinophilic focus (i)								
-minimal	0	0	0	0	0	0	1	2
-slight	0	2	0	2	0	1	0	8
-moderate	0	0	0	1	0	2	0	8
-Severe	0	0	0	0	0	0	0	1
Total	0	2	0	3	0	3	1	19
Focal cystic degeneration								
-minimal	0	3	0	6	0	9	0	9
-slight	0	7	3	8	2	12	0	14
-moderate	0	4	0	3	0	7	0	3
-severe	0	1	0	0	0	0	0	4
Total	0	15	3	17	2	28	0	30
<u>Thyroid</u>								
Intra-epithelial deposition of brown pigment								
-minimal	1	0	0	0	0	0	3	7
-slight	0	0	0	0	0	0	13	23
-moderate	0	0	0	0	0	0	2	8
Total	1	0	0	0	0	0	18	38
Hypertrophy of follicular epithelium								
-not assessable	0	8	0	7	0	9	0	5
-minimal	6	10	5	8	5	7	1	4
-slight	3	10	3	9	7	15	17	8
-moderate	0	3	0	4	0	2	0	22
-severe	0	2	0	0	0	0	0	2
Total	9	25	8	21	12	24	18	36
Depletion of colloid								
not assessable	0	8	0	7	0	9	0	5
-minimal	6	10	5	8	5	7	1	4
-slight	3	10	3	9	7	15	17	8
-moderate	0	3	0	4	0	2	0	22
-severe	0	2	0	0	0	0	0	2
Total	9	25	8	21	12	24	18	36
Focal hyperplasia								
-follicular cell hyperplastic focus(i)	0	2	0	2	0	4	1	9

I = interim kill; T = decedent + terminal kill.

Data extracted from Table 4.1, pages 197-229 of the report.

Table 8. Incidence of Significant Microscopic Findings in Females								
Parameter	Dose Level (ppm)							
	0		32		400		5000	
	I	T	I	T	I	T	I	T
No. of animals	19	51	19	51	20	50	20	50
<u>Liver</u>								
Hypertrophy of centrilobular								
minimal	0	0	0	0	0	0	1	6
Eosinophilic focus (i)								
-minimal	0	2	0	0	0	0	0	3
-slight	0	3	0	8	0	4	2	4
-moderate	0	2	0	0	0	0	0	5
Total	0	7	0	8	0	4	2	12
Focal cystic degeneration								
-minimal	0	0	0	0	0	1	0	0
-slight	0	0	0	2	0	0	0	1
Total	0	0	0	2	0	1	0	1
<u>Thyroid</u>								
Intra-epithelial deposition of								
brown pigment								
-minimal	1	0	0	0	0	0	1	1
-slight	0	0	0	0	0	0	11	28
-moderate	0	0	0	0	0	0	7	18
Total	1	0	0	0	0	0	19	47
Hypertrophy of follicular								
epithelium								
-not assessable	0	4	0	0	0	1	0	4
-minimal	4	6	5	6	4	6	3	0
-slight	2	8	0	12	1	6	10	17
-moderate	0	1	0	1	0	1	0	18
-severe	0	0	0	0	0	0	0	3
Total	6	15	5	19	5	13	13	38
Depletion of colloid								
-not assessable	0	4	0	0	0	1	0	4
-minimal	2	5	5	6	1	6	2	0
-slight	4	8	0	12	5	6	11	17
-moderate	0	1	0	1	0	1	0	18
-severe	0	0	0	0	0	0	0	3
Total	6	15	5	19	6	13	13	38
Focal hyperplasia								
-follicular cell								
hyperplastic focus(i)	0	1	0	0	0	1	0	7

I = interim kill; T = decedent + terminal kill

Data extracted from Table 4.2, pages 230-255 of the report.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosages tested in this study were adequate (although it could have been higher in male rats) for a combined chronic toxicity and carcinogenicity study based on an observation of an overall reduction in body weight gains in animals given 5000 ppm (5% in males and 42% in females). During a range-finding study at doses of 0, 80, 800, or 8000 ppm, the high dose (8000 ppm) caused a 30% reduction in body weight gains of both sexes. The NOEL was estimated to be 400 ppm (equivalent to 17 mg/kg/day for males and 22 mg/kg/day for females). The LOEL was estimated to be 5000 ppm (equivalent to 221 mg/kg/day for males and 291 mg/kg/day for females).

E. Additional Toxicology Data on Pyrimethanil

1. Mechanistic studies

The registrant has submitted additional mechanistic toxicology studies for pyrimethanil as they relate to the hypothesis that the thyroid follicular cell adenoma detected in the rat chronic study was due to hormonal imbalance secondary to liver enzyme induction.

Reference: Healing, G.: T39, T39 Addendum#1- SN 100309: Rat 7-day Dietary Thyroid Function Test Using Perchlorate Discharge as a Diagnostic Test. November 12, 1992. MRID# 43854203. Study#: TOX/92/223-55, Test facility: AgrEvo UK Limited, England.

Groups of rats (6 males/group) were fed either a control diet or diets containing 5000 ppm technical SN 100 309 (509 mg/kg/day), 2000 ppm propylthiouracil (177 mg/kg/day), or 1000 ppm phenobarbital (109 mg/kg/day) for 7 days. On Day 8, animals were injected (ip) with radiolabelled ^{125}I ; 6 hours later, all animals were killed exactly 2.5 minutes after ip injection of either saline or potassium perchlorate. There were no mortalities during the study. Animals treated with SN 100 309 had no adverse clinical effects. Propylthiouracil treated rats showed reduced activity and piloerection while Phenobarbital treatment resulted in reduced activity, unsteady gait, reduced muscle tone, piloerection, and wasted body condition. Group mean body weights of animals treated with either SN 100 309 or propylthiouracil were lower than controls at Day 4, 7, and 8 and total body weight gains (19.6% and 12.2%, respectively) were reduced compared to the control (35.4%). The food consumption was decreased by 36% at day 3 in animals treated with SN 100 309 compared with the control. Reduced food consumption in rats treated with propyl-

thiouracil was seen at days 3 and 7 (-46% and -26%, respectively). ^{125}I uptake was significantly increased in the thyroid of animals treated with either SN 100 309 or phenobarbital (150% or 221% of the corresponding concurrent saline controls, respectively). There was no significant discharge of ^{125}I after perchlorate administration in either the phenobarbital or SN 100 309 treated group. The propylthiouracil treated group showed a significant reduction in ^{125}I uptake (65% of the control), and a significant discharge of ^{125}I after perchlorate administration (Table 9). At necropsy, increased thyroid weights (+76%) and relative thyroid/body weight ratio (+113%) were seen in animals treated with propylthiouracil when compared with the control (Table 10). The results indicated that findings in animals treated with technical SN 100 309 were similar to those obtained with treatment of phenobarbital and different from propylthiouracil.

Table 9. Thyroid Count for ^{125}I Uptake and Discharge				
Test Group	+ saline		+ perchlorate	
	cpm x 1000	% uptake	cpm x 1000	% discharge
Control	86 \pm 28.7	100%	99 \pm 20.5	-15%
SN 100 309	129* \pm 21.3	150%	159** \pm 45.0	-23%
Phenobarbital	190** \pm 45.5	221%	188** \pm 70.6	1%
Propylthiouracil	56* \pm 14.7	65%	22*** \pm 8.3	61%

Data were extracted from Table 2.1, pages 27-30 of the report.
Mean \pm SD; * p = 0.05; ** p = 0.01; *** p = 0.001

Table 10. Group Mean Thyroid Weights (g) and Thyroid/Body Weight Ratios (%)			
Test Group	Body weight (g)	Thyroid weight (g)	Thyroid/ body weight ratio (%)
Control	207.5 ± 11.28	0.017 ± 0.006	0.008 ± 0.0029
Control	209.3 ± 13.25	0.016 ± 0.0034	0.008 ± 0.0012
SN 100 309	190.3 ± 7.31	0.017 ± 0.0012	0.009 ± 0.0007
SN 100 309	187.5 ± 7.97	0.015 ± 0.0019	0.008 ± 0.0011
Propylthiouracil	177.7 ± 11.44	0.030** ± 0.0037	0.017** ± 0.0026
Propylthiouracil	170.9 ± 15.95	0.029* ± 0.0078	0.017** ± 0.0039
Phenobarbital	209.8 ± 13.15	0.022 ± 0.0052	0.010 ± 0.0027
Phenobarbital	208.5 ± 9.73	0.019 ± 0.0043	0.009 ± 0.0016

Data extracted from Table 3.1-3.2, pages 31-32 of the report.
Mean ± SD; * p = 0.05, ** p = 0.01, *** p = 0.001

Reference: Healing, G.: T38 SN 100309: Rat 14-Day Dietary Study to Investigate the Mechanism of Thyroid Response. November 12, 1992. MRID# 43854202. Study# TOX/92/223-56. Test facility: AgrEvo UK Limited, England.

Technical SN 100 309 was administered in a diet to rats (10 males/group) for 14 days at a dosage of 0 or 5000 ppm (equivalent to 378.5 mg/kg/day). There were no mortalities or treatment-related clinical signs reported. A reduction in body weight gain (23%) was seen in treated rats at the first week only. Rats treated with 5000 ppm SN 100 309 for 14 days showed a markedly increased liver enzyme UDPGT level (446% of the control) (Table 11). Also, significantly increased TSH levels were seen at Days 1, 4, and 15 of the study (162%, 155%, and 215% of respective controls) while decreased T3 and T4 levels (82% and 76% of respective controls) were noted at Day 4. Liver weights of treated rats were significantly higher (23%) than controls at the interim kill while the thyroid weight was significantly lower (56%) than the control (Table 12). After a 14-day off-dose period, liver weight and plasma T3, T4, TSH, and RT3 levels were back to normal while the UDPGT level remain elevated (163% of the control). Histopathologically, 14 days of treatment with SN 100 309 resulted in centrilobular hepatocyte enlargement in the liver and colloid depletion, follicular cell hypertrophy and follicular epithelial hyperplasia in the thyroid gland. Following a 14-day off-dose period, these findings were back to normal.

Table 11. Findings of hormones and liver assays						
Day	Dose (ppm)	T3 (nmol/L)	T4 (nmol/L)	TSH (ng/mL)	RT3 (pmol/L)	UDPGT (U/L/mg)
6 hr	0	1.7 ± 0.2	107 ± 16.9	6.6 ± 1.2	20.3 ± 1.8	----
	5000	1.4 ± 0.2	91 ± 12.7	10.2 ± 7.4	21.7 ± 6.0	----
24 hr	0	1.3 ± 0.3	88 ± 19.9	6.0 ± 0.8	17.3 ± 2.8	----
	5000	1.1 ± 0.2	85 ± 3.9	9.7** ± 2.2	20.9* ± 1.4	----
Day 4	0	1.1 ± 0.3	84 ± 18.5	8.3 ± 2.0	16.3 ± 2.7	----
	5000	0.9* ± 0.2	64** ± 9.7	12.9* ± 4.9	20.0 ± 5.0	----
Day 8	0	1.3 ± 0.1	76 ± 16.0	10.2 ± 3.7	14.8 ± 3.2	----
	5000	1.2 ± 0.1	75 ± 16.5	14.0 ± 7.9	17.8 ± 2.9	----
Day 15	0	1.0 ± 0.2	69 ± 13.8	7.2 ± 2.5	15.6 ± 5.5	71 ± 24.4
	5000	1.0 ± 0.1	78 ± 15.7	15.5** ± 7.4	17.8 ± 3.8	317*** ± 48.7
Day 29	0	1.1 ± 0.2	65 ± 16.6	9.5 ± 1.9	13.8 ± 3.0	41 ± 11.4
	5000	1.2 ± 0.2	80 ± 4.9	8.8 ± 3.4	15.2 ± 1.9	67** ± 11.0

Data extracted from Table 2.1, pages 31-36 of the report.
Statistical significance: * p = 0.05; ** p = 0.01; *** p = 0.001

Table 12. Organ Weight and Organ/Body Weight Ratios						
	Dose (ppm)	Liver		Thyroid		Body Weight (g)
		Absolute wt (g)	L/B ratio (%)	Absolute wt (g)	T/B ratio (%)	
Interim	0	12.81 ± 1.428	3.63 ± 0.466	0.043 ± 0.0066	0.012 ± 0.0021	353.4 ± 12.8
	5000	15.81* ± 2.259	4.74** ± 0.337	0.019** ± 0.0029	0.006** ± 0.0013	331.9 ± 27.5
Terminal	0	15.44 ± 2.052	3.63 ± 0.377	0.033 ± 0.0034	0.008 ± 0.0012	426.2 ± 46.6
	5000	13.92 ± 1.521	3.42 ± 0.194	0.019** ± 0.0044	0.005** ± 0.001	407.6 ± 40.3

Data were extracted from Table 3.1-3.2, pages 37-40 of the report.
L/B ratio= Liver/body weight ratio; T/B ratio= Thyroid/body weight ratio.

Data were presented as Mean ± SD; * = p ≤ 0.05; ** = p ≤ 0.01.

2. Metabolism

The majority of the administered pyrimethanil was eliminated within 24 hours via the urine and feces following a single oral dose of 11.8 mg/kg or 800 mg/kg; radiolabel was detected in the liver, kidney and blood at 24 hours. The highest residue was displayed in the liver in both sexes. The main

pathways of metabolism involved oxidation to phenols in either or both aromatic rings, and the minor pathways involved oxidation of the methyl group to the corresponding alcohol. Minor differences were observed between the high- and low-dose exposures, the single low- and repeat low-dose exposures, and between the sexes, but none appear to be of any toxicological significance.

Summaries of metabolism studies are as follows.

Reference: Hemmings, PA: (M9) PYRIMETHANIL (SN 100309): Excretion and Tissue Residues of a Radiolabelled Oral Dose in Rats Following Pre-Dosing for 14 Days with Unlabelled SN 100309. May 21, 1995. MRID# 43750101; Doc# 011695. Study# TOX/90299. AgrEvo UK Ltd. England.

The majority [~90%] of the administered dose of ¹⁴C-SN 100 309 [>99%] following 14 days of repeated oral exposure to unlabeled SN 100 309 [99.4%] to Sprague-Dawley Crl:CD(SD)BR rats [5/sex] at a dose level of 10 mg/kg/day was eliminated within 24 hours, and the major route of elimination was via the urine [~72%]. Approximately 17-18% of the dose was eliminated via the feces. Radiolabel was detected only in the liver, kidney, and blood at study termination [24 hours post dose]. The highest residue was displayed in the liver in both sexes. There was no significant sex difference. The overall recovery of radiolabel was ~91%.

Reference: Hemmings, PA: (M1) SN 100 309: Residue Levels in Rat Tissues Following Repeated Daily Oral Dosing with [¹⁴C]-SN 100 309 at 10 mg/kg Body Weight. July 25, 1991. MRID# 43301629; Doc# 011695. Study# TOX/90300. Schering Agrochemicals Ltd., England.

Rats [strain not identified; 3 males/group] were administered [¹⁴C]-SN 100 309 [>98%] orally once a day over a period of 28 days [10 mg/kg/day], with periodic sacrifices at days 1, 3, 5, 8, 11, 17, 23, 28, and 32 for residue analysis of organs/tissues. Detectable levels of radiolabel were found in the adrenals, blood, kidney, liver, spleen, and thyroid following exposure. Only the blood and liver displayed detectable levels of radiolabel after a single dose [24-hour sample]. Four days after the last dose, detectable levels of radiolabel were found only in the liver, kidney, and thyroids. It appears that the levels in the blood, kidney, and thyroid would continue to increase with increased exposure time, while the level in the adrenal appears to have reached a plateau, and that in the liver appeared to be declining.

Reference: Needham, D. and Hemmings, PA: (M5/2) SN 100 309: Metabolism in the Rat. August 2, 1993; MRID# 43345009. Doc# 011695 Study# TOX/89373/5AN. Schering Agrochemicals Ltd. England.

SN 100 309 [pyrimethanil] was shown to be metabolized extensively in the CrI:CD(SD)BR rat [both sexes] following single oral doses of ^{14}C -pyrimethanil at 11.8 mg/kg or 800 mg/kg or 14 consecutive daily oral doses of unlabeled pyrimethanil at 10 mg/kg/day followed by a single [10 mg/kg] oral dose of ^{14}C -pyrimethanil. None of the pyrimethanil was found in the urine following any exposure regimen. In the feces, small amounts of pyrimethanil [$\approx 6\%$, $\approx 4\%$, and $\approx 11\%$ of total radiolabel in the low-, repeat low-, and high-dose rats, respectively] were found following all exposures. The main pathways of metabolism involved oxidation to phenols in either or both aromatic rings, and the minor pathways involved oxidation of the methyl group to the corresponding alcohol. Minor differences were observed between the high- and low-dose exposures, the single low- and repeat low-dose exposures, and between the sexes, but none appear to be of any toxicological significance.

Reference: (M2) The Distribution and Excretion of Radiolabelled Residues in the Rat Following Oral Dosing with SN 100 309 at 11.8 or 800 mg/kg body Weight. (Study# TOX/89372/90293, dated August 22, 1991; MRID# 43301630; Doc# 011695).

The majority [$\approx 97\%$ low dose; $\approx 65\%$ high dose] of the administered dose of radiolabelled SN 100 309 following single oral exposure to rats [Sprague-Dawley CrI:CD(SD)BR; 5/Sex] at dose levels of 11.8 mg/kg or 800 mg/kg was eliminated within 24 hours, and the major route of elimination was via the urine [low dose 74%-76%; high dose 65%-67%]. Approximately 21%-23% of the low dose was excreted via the feces, and $\approx 15\%$ -18% of the high dose was eliminated via the feces. Radiolabel was detected only in the liver and carcass following low dose exposure but was detected in nearly all tissues [exceptions pituitary (all), eyes (4/5), and renal fat (2/5)] measured following the high dose. The concentration of radiolabel remaining in the liver [only organ for comparison] at 96 hours was dose-related; i.e., the levels of radiolabel in the liver at the two dose levels were proportion to the dose. The highest residues were displayed in the liver, kidney, thyroid, and blood at the high dose. The overall recovery of radiolabel following single-dose exposure was $>94\%$ at the high dose and $>101\%$ at the low dose. No sex differences were

observed. Since tissue levels were measured at only one time point, no statement regarding bioaccumulation can be made.

3. Mutagenicity

Reference: (a) (T14) SN 100 309: Bacterial Mutation Assay [Study# SMS 185/90509, dated July 19, 1990; MRID# 43301624]. (b) (T21) Technical SN 100 309: Mouse Micronucleus Test [Study# SMS 256/901828, dated May 20, 1991; MRID# 43301626]. (c) (T15) Metaphase Chromosome Analysis of human lymphocytes Culture in vitro [Study# SMS 203/90416, dated July 23, 1990; MRID# 43301627]. (d) (T24) Technical SN 100 309: Bacterial Mutation Assay with *Escherichia coli* [Study# SMS 303/92489, dated September 2, 1991; MRID# 43301625]. (e) (T22) Technical SN 100 309: Unscheduled DNA Synthesis Assay in Rat Hepatocytes Treated in vivo [Study# SMS 258/9171, dated June 21, 1991; MRID# 43301628].

a. *Salmonella*/mammalian activation gene mutation assay. Negative for inducing reverse gene mutation with *Salmonella typhimurium* strains TA1535, TA 1537, TA 1538, TA 98 and TA 100 with or without metabolic activation; pyrimethanil levels up to 1500 µg/plate.

b. In vivo mammalian cytogenetics-micronucleus assay in mouse. Negative for the induction of micronuclei in the bone marrow cells of male and female CD-1 mice at 900 mg/kg.

c. In vitro mammalian cytogenetics - chromosomal analysis of cultured human lymphocytes assay. Negative for increased incidence in chromosomal aberrations associated with exposure to SN 100 309 at dose levels of 7.8 µg/ml to 250 µg/ml without S9 and 31.1 µg/ml to 250 µg/ml with S9.

d. *E. coli* bacterial mutation assay. Negative in increased incidence of tryptophan revertants as a result of exposure to SN 100 309 at dose levels up to 1500 µg/plate.

e. Unscheduled DNA synthesis in primary rat hepatocytes treated in vivo. Negative in inducing unscheduled DNA synthesis in rat hepatocytes as a result of in vivo gastric intubation of SN 100 309 at dose levels of 100, 300, and 1000 mg/kg.

The above studies (performed before 1991) satisfy the pre-1991 mutagenicity guideline for a test in the three categories of gene mutation, structural chromosomal aberrations, and other genotoxic effects. Based on these data, there is no concern for mutagenicity.

4. Structure Activity Relationship

Pyrimethanil is a new chemical. No suitable analogs were found in the Tox data base.

5. Acute, Subchronic, and Chronic Toxicity

Acute Toxicity

Guideline	MRID#	Type of Study	Results	Tox Cat.	Core Grade
81-1	43345002	Acute Oral- Rat	LD50= 4149 mg/kg (♂) 5971 mg/kg (♀)	3	Acceptable
81-2	43345003	Acute dermal- Rat	LD50 >5000 mg/kg	4	Acceptable
81-3	43301604	Acute Inhalation-Rat	LC50 >1.98 mg/L	N/A	Supplementary (upgradable)
81-4	43345004	Eye Irritation- Rabbit	Slight eye irritant	4	Acceptable
81-5	43345005	Skin Irritation- Rabbit	Non-irritant	4	Acceptable
81-6	43301605	Dermal sensitization- Guinea pig	Not skin sensitizer	N/A	Supplementary (upgradable)

Subchronic Toxicity

(T13) SN 100 309: 13-Week Oral (Dietary) Toxicity Study in the Rat Followed by a 4 Week Regression Period - AT Higham, Toxicol Laboratories Limited; dated November 13, 1990. (MRID# 433450-06) and (T13 amend 1) - PW Harvey, Schering Agrochemical Ltd. dated October 20, 1992. (MRID# 433016-08)

For a 90-day study, groups of Sprague Dawley CD rats (10/sex/group) were fed diets containing SN 100 309 at dose levels of 0, 80, 800, or 8000 ppm for 13 weeks. Those doses were equivalent to daily intake of 0, 5.4, 54.5, 529.1 mg/kg/day for males and 0, 6.8, 66.7, 625.9 mg/kg/day for females, respectively. A supplementary control and a high dose (8000 ppm) groups (10/sex/group) were similarly treated for 13 weeks then maintained off-dose for 28 days to investigate the reversibility of any findings. Treatment of SN 100 309 did not affect mortality, clinical signs, water intake, ophthalmology, hematology, blood chemistry or macroscopic pathology. At 8000 ppm, mean body weight gains were 28% lower in males and 33% lower in females than controls over the whole treatment period. The decreased body weight gains were consistent with reduced food consumptions in animals given 8000 ppm in which a

reduction of 33% in the first week and approximately 18% for the remainder of the study were noted. Urinalysis at the end of the treatment period showed dark brown coloration of urine in both sexes. An increase of urinary protein was reported in males only. Significantly lower absolute organ weights were seen in the heart and adrenal glands of males, and in the heart, spleen, thymus, kidneys, and adrenal glands of females. Significant increases of relative organ to body weight ratios were seen in the brain, liver, gonads, and kidneys for males, and in the brain, liver, and kidneys for females. Microscopic pathology showed an increased incidence in hypertrophy of centrilobular hepatocytes in both sexes (9/10 in males and 3/10 in females). In the thyroid, treatment-related increases in incidence and severity of follicular epithelial hypertrophy (9/10 in males and 6/10 in females) and follicular epithelial brown pigment (8/10 in males and 7/10 in females) were observed. Following a 28-day reverse period, the incidence of hypertrophy of follicular epithelium were 6/10 for males and 3/10 for females compared with 4/10 for controls. Brown pigment in the epithelium was observed in treated animals (2/10 in both sexes). At 800 ppm, significant increases of organ to body weight ratios of liver and kidney were seen in females only. The results were associated with decreased body weight gains in this treatment group. Changes in the coloration of urine specimens and an increase of incidence (2/10) in hypertrophy of centrilobular hepatocytes were observed in males only. At 80 ppm, no treatment-related findings were observed for either sex. During the reversibility period, animals previously given 8000 ppm showed evidence of recovery. However, the total body weights at the end of the 28-days reversibility period were still lower than controls (15% in males and 13% in females). Under the conditions of this study, the **No Observed Effect Level (NOEL)** was estimated to be 80 ppm (equivalent to daily intake of 5.4 mg/kg/day for males and 6.8 mg/kg/day for females). The **Lowest Observed Effect Level (LOEL)** was estimated to be 800 ppm (54.5 mg/kg/day for males and 66.7 mg/kg/day for females). The LOEL is based on decreased body weight gains in females, changed coloration of urine specimens and increased incidence of hypertrophy of centrilobular hepatocytes in males.

(T17) SN 100 309 (CR 19325/3): Mouse 90-day Dietary Repeat Dose Study - PW Harvey and SJ Rees, Schering Agrochemical Ltd.; dated November 4, 1991. (MRID# 433016-06)

In a 90-day feeding study, groups of CD-1 mice (20/sex/group) were fed diets containing technical SN 100 309 (97.7% - 97.9%) at dose levels of 0, 80, 900, or 10000 ppm for 13 weeks. Those doses were equivalent to 0, 12, 139 or 1864 mg/kg/day for

males and 0, 18, 203 or 2545 mg/kg/day for females, respectively. There were no treatment-related effects in mortality, clinical signs, water intake, or hematological parameters. At 10000 ppm, total body weight gains were 12.4% lower in males and 7.2% lower in females than controls. There was an overall increase in food consumption (14.1% in males or 9.8% in females) compared with controls, resulting in reduced food conversion ratios in treated animals. Clinical chemistry parameters showed significant increases in serum cholesterol and total bilirubin (in females only). Treatment-related necropsy findings included dark thyroid glands in 8/10 males and a bladder stone in 1/10 females. Significantly increased liver weight in females and increased relative liver to body weight ratios in both sexes were noted in animals given 10000 ppm. Histopathological changes were detected in the kidneys, liver, thyroid glands, and urinary bladder. Tubular dilation was seen in the kidneys of 3/10 males. Uroliths were detected in urinary bladders of 1/10 males and 4/10 females (one bladder stone was detected at necropsy), with 3/10 females showing hyperplasia of the bladder epithelium at the dose level of 10000 ppm. There was a marked depletion of glycogen in the liver of both sexes indicated by decreased margination of cytoplasm and reduced PAS staining intensity (done in males only). In the thyroid gland, exfoliative necrosis of follicular cells was seen in 8/10 males and 1/10 females. Pigmentation of follicular cells was seen in 10/10 males and 9/10 females at 10000 ppm. Special staining techniques indicated that the pigment was lipofuscin. At 900 ppm, some glycogen depletion was still evident in the liver; however, no significant histopathological finding was reported at this dose. This condition is associated with the nutritional and/or physiological status of the animals and has no toxicological significance. At 80 ppm, no treatment-related effects were detected. The No Observed Effect Level (NOEL) was estimated to be 900 ppm, equivalent to daily intake of 139 and 203 mg/kg/day for males and females, respectively. The Lowest Observed Effect Level (LOEL) was estimated to be 10000 ppm, equivalent to daily intake of 1864 and 2545 mg/kg/day for males and females, respectively. The LOEL is based on decreased body weight gains, clinical chemistry data, necropsy and histopathological findings.

(T16) Technical SN 100 309: Dog 90-day Oral (Gavage) Repeat Dose Study - PW Harvey, Schering Agrochemical Ltd.; dated October 10, 1991. (MRID# 433016-10)

For a 90-day study, groups of outbred beagle dogs (4/sex/group) received technical SN 100 309 by gavage at dose levels of 0, 6, 80, or 1000/800 mg/kg/day for 13 weeks. The

highest dose was reduced from 1000 mg/kg/day to 800 mg/kg on day 7 due to persistent vomiting seen in all dogs receiving 1000 mg/kg. Concentrations of dosing suspensions were within ranges of 82.5% to 121.7% of nominal. The following parameters were examined: mortality, clinical signs, body weights, food and water consumption, hematology, clinical chemistry, gross pathology, organ weights, and microscopic pathology. There were no treatment-related effects on mortality, organ weights, necropsy findings, histopathological, ophthalmoscopical, or hematological parameters. At 1000 mg/kg, there was an increased incidence of vomiting and diarrhea in all animals during the first 6 days of treatment resulting in slight body weight losses (~4%). Food consumption was reduced in both sexes (23% in males, 16% in females compared with controls) during the first week of treatment. When the dose was reduced to 800 mg/kg occasional to frequent vomiting (approximate 9% of all doses) was observed. Food consumption was comparable to controls in both sexes. The overall water consumption was markedly decreased in both sexes (30% in males, 19% in females compared with controls). Other clinical signs included salivation, diarrhea, cream or red coloration of feces, and hypoactivity. Clinical chemistry analysis indicated that there were significant reductions in serum phosphate level in males and serum total protein in females after 4 weeks treatment. At 80 mg/kg, infrequent vomiting (less than 2% of all doses) was observed. The water consumption was decreased (9% in males and 17% in females). Other clinical signs included salivation, diarrhea, cream coloration of feces, and hypoactivity. Clinical chemistry analysis indicated that there was a small reduction in serum phosphate level in males at week 4 and in females at week 1. At 6 mg/kg, no treatment-related effects were reported. Under the conditions of this study, the **No Observed Effect Level (NOEL)** was estimated to be 6 mg/kg. The **Lowest Observed Effect Level (LOEL)** was estimated to be 80 mg/kg. The LOEL is based on the increased incidence of vomiting and diarrhea, salivation, cream coloration of feces, hypoactivity, and decreased water consumption.

Chronic Toxicity

(1) (T26/2) Technical SN 100 309: Dog 12 Month Oral (Gavage) Repeat Dose Study - SJ Rees, Schering Agrochemical Ltd.; dated November 11, 1992. (MRID# 433450-07) (2) (T26A) Determination of SN 100 309 Suspension Concentrations for a 12 Month Study in the Dog - M Crofts, Schering Agrochemical Ltd.; dated September 4, 1992. (MRID# 433016-14)

In a one-year oral toxicity study, Beagle dogs [4/sex/dose] were administered SN 100 309 technical (96.3% to 96.9% a.i.)

by oral gavage at doses of 0, 2, 30, or 400/250 mg/kg/day for 12 months. Administration of the test material at 400 mg/kg/day caused a high incidence of vomiting/emesis [35.7% in 4/4 males and 3/4 females) during Week 1 of the study. For this reason, the dose regimen was decreased to 250 mg/kg/day on Day 8 of the study. At this dose (250 mg/kg) vomiting was decreased to about 1% in all animals. Overall mean body weight gain at the high dose (400/250 mg/kg) was reduced by 50% in males and 73% in females when compared to the controls. Overall food conversion efficiency was reduced in males and females by about 50% and 98%, respectively. Overall water consumption at 400/250 mg/kg was significantly decreased by 35% and 26% in males and females, respectively. A statistically significant increase in white blood cells, primarily neutrophils, was observed in males at 400/250 mg/kg after 3, 6 and 12 months of treatment. In both males and females, clotting times (Thrombotest) were significantly reduced after 12 months. There were no treatment-related findings on histopathology. There were no significant treatment-related effects at 2 or 30 mg/kg. Based on the results of this study, the no observed effect level (NOEL) is 30 mg/kg/day and the lowest observed effect level (LOEL) is 250 mg/kg/day, based on decreases in body weight, food consumption, feed efficiency, and water consumption, reduced clotting times and increases in white blood cells, (mainly neutrophils).

F. Weight of Evidence Considerations

The Committee considered the following facts regarding the toxicology data on pyrimethanil in the Weight-of-the-Evidence determination of its carcinogenic potential:

1. In the mouse carcinogenicity study (MRID 43301615), male and female CD-1 mice were fed 0, 16, 160, or 1600 ppm of pyrimethanil (equivalent to 0, ♂2.0/♀ 2.5, ♂ 20.0/♀ 24.9, or ♂ 210.9/♀253.8 mg/kg/day, respectively) for 80 weeks. There were no adverse effects on general health, body weight, body-weight gain, food or water consumption, differential WBC counts, organ weights, or tumor incidence in either sex. Survival was comparable among the female groups, but males at all dose levels displayed an increase in the number of deaths due to urogenital tract lesions compared to controls. Although there were increases in the number of deaths in the treated males relative to the control, there were adequate numbers of mice at each dose level at termination for an assessment of the carcinogenic potential. However, it appears that a higher dose level could have been tolerated. The CPRC noted that the body weight gains in males of the highest dose group was only 5% lower than the control group; in addition, the incidence of

stones was low in males [1/10] and moderate [4/10] in females in the 90-day study, and the other urinary bladder lesions [uroliths in lumen, urothelial hyperplasia] also occurred at the 10000 ppm dose only. The next highest dose level used in the 90-day study was 900 ppm, a dose at which no lesions were observed. The CPRC concluded that the dosages for the mouse carcinogenicity were inadequate.

2. In the combined chronic toxicity/carcinogenicity study (MRID 43301612), male and female Sprague-Dawley rats were fed 0, 32, 400, or 5000 ppm of pyrimethanil (equivalent to 0, ♂ 1.3/♀ 1.8, ♂ 17/♀ 22, ♂ 221/♀ 291 mg/kg/day, respectively) for 104 weeks. Male rats had significant increasing trends for thyroid follicular cell adenomas and adenomas and/or carcinomas combined, both at $p < 0.01$. There was a significant difference in the pair-wise comparison of the 5000 ppm dose group with the controls for thyroid follicular cell adenomas and/or carcinomas combined at $p < 0.05$. There was also a significant increasing trend for thyroid follicular cell adenomas in the interim sacrifice group at $p < 0.05$ by the Exact Trend Test. Female rats had a significant increasing trend, and significant differences in the pair-wise comparisons of the 32 and 5000 ppm dose groups with the controls, for thyroid follicular cell adenomas, all at $p < 0.05$. The CPRC concluded that the test dosages for this study were adequate (although it could have been higher in male rats) and that pyrimethanil causes compound related thyroid tumors in Sprague-Dawley rats of both sexes.

3. Pyrimethanil was negative in the *Salmonella*/mammalian activation gene mutation assay, *in vivo* mammalian cytogenetics-micronucleus assay in mouse, *in vitro* mammalian cytogenetics-chromosomal analysis of cultured human lymphocytes assay, *E. coli* bacterial mutation assay, or unscheduled DNA synthesis (UDS) assay.

4. There were no suitable analogs to propose a structure-activity co-relationship.

5. The CPRC found the mechanistic studies were acceptable and considered the use of the threshold model for pyrimethanil.

6. Consideration of the Use of the Threshold Model for Pyrimethanil

When evaluating pyrimethanil, the Committee considered the possibility of using the threshold model for thyroid neoplasms. The following discussion has been taken from the Amitrole Peer Review Document and revised for pyrimethanil.

The following guidance is given in the Agency's DRAFT Policy Document (Thyroid Follicular Carcinogenesis: Mechanistic and Science Policy Considerations, SAB Review Draft, May 1988):

"Studies over the last several decades in multiple laboratories and using a number of different treatment regimens (eg., iodine deficiency) have demonstrated the significance of long-term thyroid-pituitary hormonal imbalance in thyroid carcinogenesis. A consistent progression of events is noted: reduction in thyroid hormone concentrations, elevation in TSH levels, cellular hypertrophy and hyperplasia, nodular hyperplasia, and neoplasia. Hyperplasia and sometimes neoplasia of the pituitary may also be seen.. A block in any of the early steps acts as a block for subsequent steps including tumor development, and cessation of treatment at an early stage in the progression results in regression toward normal thyroid structure and function. Based on these observations the Agency concludes that:

- a. thyroid follicular cell tumors may arise from long-term disturbances in thyroid-pituitary feedback under conditions of reduced circulating thyroid hormone and elevated TSH levels:
- b. the steps leading to these tumors are expected to show thresholds, such that the risks of tumor development are minimal when thyroid-pituitary homeostasis exists; and
- c. models that assume thresholds may be used to assess the risks of thyroid follicular cell tumors where there is evidence of thyroid-pituitary hormonal imbalance."

Two basic questions must be addressed before this policy is applied.

"The first is a qualitative issue which addresses whether it is reasonable to presume that the neoplasms are due to thyroid-pituitary imbalance. A corollary issue is the extent to which other carcinogenic mechanisms can be discounted. The second question concerns the procedures to be employed in estimating the risks of these agents."

"The answers to the first question allow one to assign chemicals producing thyroid tumors to one of three categories. The assignation is based upon knowledge as to whether the chemical disrupts thyroid-pituitary feedback, whether tumors other than thyroid follicular cell (and relevant pituitary) tumors are found, and whether mechanisms other than thyroid-pituitary imbalance may apply to the observed tumor response."

Determination of whether neoplasms are due to thyroid-pituitary imbalance

The document goes on to describe the 3 factors which should be considered in making this determination (answering the first question, or "qualitative issue"). These are addressed as they apply to pyrimethanil as follows:

FACTOR I. Consideration of whether the thyroid tumors associated with administration of pyrimethanil can be attributed to disruption of the thyroid-pituitary hormonal balance. (In addressing this factor, the Policy states, 6 indicators should be considered.)

a. Goitrogenic activity in vivo:

Thyroid follicular cell hyperplasia and/or hypertrophy were observed in the highest dose of males and females in the chronic rat study. In addition, these effects were also observed in the 90-day feeding study and in the special thyroid function study in rats of both sexes at high doses.

b. Clinical chemistry changes (eg., reduced thyroid hormone and increased TSH serum concentrations):

In a chronic toxicity/carcinogenicity study, thyroid hormone levels were normal when sampled during the week 72. However, in special thyroid function studies in rats, after 6 hours of treatment with pyrimethanil at 5000 ppm, animals had a reduced plasma T4 level and elevated plasma TSH. After 24 hours, statistically significant increases in TSH and RT3 (Reverse T3) was observed. By day 4, statistically significant reductions in T4 and T3 and a concomitant increase in TSH was seen. On day 8 and 15, group mean TSH levels were still higher than corresponding controls, although plasma thyroid hormone levels had returned to normal levels. After a 14-day period on control (off-dose) diet, a complete reversal of all toxicologically significant effects occurred.

c. Specific evidence of reduced hormone synthesis (eg., inhibited iodine uptake) or increased thyroid hormone clearance (eg., enhanced biliary excretion):

The following effects were observed in special thyroid function studies: A significant increase of ¹²⁵I uptake (150% of the control) was seen in animals treated with pyrimethanil for 7 days. In addition, an increase of liver microsomal UPDGT level (447% of controls) was seen after 14 days of pyrimethanil treatment. The enzyme levels remained higher than

controls following a 14-day recovery period.

d. Evidence of progression (eg., hypertrophy/hyperplasia, nodular hyperplasia - neoplasia):

There was evidence of progression (hypertrophy/hyperplasia to neoplasia) in rats. In special thyroid function studies, thyroid follicular cell hypertrophy and hyperplasia were seen in rats (only males were tested) treated with 5000 ppm of pyrimethanil for 14 days. In the 90-day feeding study, thyroid hypertrophy was observed at the 8000 ppm group (HDT) of both sexes. In the two-year rat study, the first thyroid hypertrophy was observed in both sexes at week 52 and the first hyperplasia was observed in a male (1/20) of the 5000 ppm dose group at week 52. Thyroid follicular cell adenomas were first observed in an interim sacrifice of both sexes (week 53 for the female and week 54 for the male) at 5000 ppm dose group. The first adenocarcinoma was observed in the male of the low-dose group (32 ppm) at week 107. Significant increases in thyroid follicular cell tumors were evident in males and females by the end of the study.

e. Reversibility of effects after exposure is terminated:

In the special thyroid function study, with the exception of liver microsomal UPDGT level, all of the following effects were shown to be reversible or nearly reversible upon removal of pyrimethanil in the diet: serum T4, T₃, RT3, TSH concentrations, and thyroid follicular cell hypertrophy/hyperplasia.

f. SAR to other thyroid tumorigens: No suitable analogs have been found.

Based on the overall judgment of the 6 indicators in Factor I, it may be concluded that there are sufficient data to determine that the thyroid tumors in the rat associated with administration of pyrimethanil may be due to a disruption in the thyroid-pituitary status.

FACTOR II. Consideration of the extent to which genotoxicity may account for the observed tumor effects.

The genotoxicity data are negative. There is no indication that genotoxicity plays a role in the tumorigenic activity for this chemical.

FACTOR III. Evaluation of neoplasms other than thyroid follicular cell tumors (and relevant pituitary tumors).

There were no statistically significant increases in any other tumor types in either rats or mice for this chemical; however, the test dosage in the mouse study may not have been adequate.

Conclusions: As indicated above, based on the overall judgment of the 6 indicators in Factor I, it may be concluded that there are sufficient data to determine whether or not there is suggestive evidence that the thyroid tumors in the rat associated with administration of pyrimethanil may be due to a disruption in the thyroid-pituitary status. Adding in Factors II and III, this conclusion still stands. All of the criteria for a threshold effect have been met.

Factors to be Considered in Determining Method to be Used in Estimating the Risks of Pyrimethanil

Again, this guidance was taken from the Amitrole Peer Review Document and revised for pyrimethanil. The Committee has considered these points when determining which method is to be used for estimating the carcinogenic risk for pyrimethanil.

Guidance given in the EPA DRAFT policy for proceeding with the quantitation of risk is as follows:

- a. "Threshold considerations should be applied in dose-response assessments for those chemical substances where (1) only thyroid tumors (and relevant pituitary tumors) have been produced; (2) the tumors can be attributed to a disruption in thyroid-pituitary hormonal homeostasis; and (3) potential mechanisms other than thyroid-pituitary imbalance (eg., genotoxicity) can be disregarded.
- b. Special attention should be given to chemicals (1) that have induced thyroid tumors (and relevant pituitary tumors) that may be due to thyroid-pituitary imbalance, and (2) where there is also evidence of either a genotoxic potential or the induction of neoplasms at sites other than the thyroid (or pituitary). Generally, those cases will be approached using various principles laid out in the EPA Guidelines for Carcinogen Risk Assessment. A strong rationale must be articulated for handling these agents otherwise.
- c. For those chemicals producing thyroid tumors that do not seem to be acting via thyroid-pituitary hormonal inhibition, dose-response assessments will be performed in accordance with

the EPA Guidelines for Carcinogen Risk Assessment."²

Based on the evidence that pyrimethanil appears to induce thyroid tumors through a disruption in the thyroid-pituitary status, and thus may have a threshold for tumor development, the Committee recommended that a Margin of Exposure (MOE) approach be used for quantitating carcinogenic risk. This decision was supported by the weight of the evidence, considering the neoplastic, related nonneoplastic and/or hormonal effects in the male rat thyroid and liver.

The selection of a NOEL for the MOE approach utilizes only those biological endpoints which are related to tumor development. Therefore, when selecting the most appropriate NOEL and LOEL to use for the carcinogenic risk assessment of pyrimethanil, the following endpoints were considered by the CPRC:

Two-year rat study

- Thyroid tumor
- Thyroid hypertrophy/hyperplasia
- Hepatocellular hypertrophy
- Increase in liver weight

90-Day rat study

- Thyroid hypertrophy
- Hepatocellular hypertrophy

All of the endpoints above that were observed either in the thyroid or liver were considered to be directly related to the thyroid neoplastic response in rats. Therefore, in light of the definition given above, all of these endpoints were considered to be appropriate for use in the selection of a NOEL and a LOEL for the carcinogenicity risk assessment on

²It is noted that a new policy document is in process which currently states these phrases differently: 1. "Threshold considerations will be incorporated into thyroid (and relevant pituitary) cancer dose-response assessments for chemicals that (a) cause disruption of thyroid-pituitary homeostasis and (b) are judged not to have genotoxic activity relevant to carcinogenicity. Dose-response relationships for neoplasms other than the thyroid (or pituitary) should be evaluated using mechanistic information bearing on their induction and various principles laid out in the Agency's cancer risk assessment guidelines. 2. Threshold considerations may be applied in thyroid cancer dose-response assessments on a case-by-case basis for chemicals that (a) produce thyroid-pituitary imbalance and (b) are judged to have genotoxic activity related to carcinogenicity. The implications of the genotoxic events to the thyroid carcinogenic responses need to be carefully evaluated. In some cases thyroid cancer dose-response relationships may be characterized in more than one way. 3. Threshold considerations will not be applied in thyroid cancer dose-response assessments for substances operating through mechanisms not involving thyroid-pituitary imbalance. However, case-by-case exceptions may arise, based on mode of action data."

pyrimethanil utilizing a M.O.E. approach.

In addition, the same endpoints were examined in other species. In the mouse carcinogenicity study, no thyroid or liver tumors were observed; however, the CPRC indicated that the test dosage for this study was inadequate.

The following table summarizes the studies, endpoints, NOELs and LOELs considered for CPRC's decision.

Factors Considered for Determining NOEL for Margin of Exposure for Pyrimethanil			
Study	Endpoint	NOEL (mg/kg/day)	LOEL (mg/kg/day)
2-Year Rat	thyroid tumors	17 (♂), 22 (♀) ^a	221 (♂), 291 (♀)
	thyroid hypertrophy/hyperplasia	17 (♂), 22 (♀)	221 (♂), 291 (♀)
	hepatocellular hypertrophy	17 (♂), 22 (♀)	221 (♂), 291 (♀)
	↑ liver weights	1.3 (♂), 22 (♀)	17 (♂), 291 (♀)
90-Day Rat	Thyroid hypertrophy	54.5 (♂), 66.7 (♀)	529.1 (♂), 625.9 (♀)
	Centrilobular hypertrophy in hepatocytes	54.5 (♂), 66.7 (♀)	529.1 (♂), 625.9 (♀)
18-Mo. Mouse	No thyroid or liver tumors reported.	210.9 (♂), 253.8 (♀) (HDT)	----
1-Year Dog	No tumors or abnormal histopathological findings reported.	250 (♂,♀) (HDT)	----
90-Day Mouse	Follicular cells necrosis	139 (♂), 203 (♀)	1864 (♂), 2545 (♀)

^aA statistical analysis in females showed a significant increase of the thyroid follicular cell adenomas in the pair-wise comparisons of the 32 ppm (1.8 mg/kg/day) and 5000 ppm (291 mg/kg/day) dose groups compared with the controls. However, the significance of the 32 ppm dose group was questionable because (1) the incidence in the control group was low (0%) compared with the historical control data (1.1%); (2) there was no dose relationship and the 400 ppm dose group did not show a significant difference from the control. Therefore, the NOEL for thyroid tumors in females was estimated to be 400 ppm (22 mg/kg/day).

For the MOE calculation, the committee selected the NOEL and LOEL which represented the majority of the observations. The NOELs were 17 mg/kg/day for males and 22 mg/kg/day for females, the LOELs were 221 mg/kg/day for males and 291 mg/kg/day for females.

For comparison, the NOELs selected for calculation of the RfD for pyrimethanil are 17 and 22 mg/kg/day for males and females, respectively, based on decreased body weight, increased serum cholesterol and GGT levels, increased relative liver/body weight ratios, as well as gross and histopathological alterations in a chronic feeding study in rats.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for pyrimethanil.

The Peer Review Committee agreed that pyrimethanil should be classified as a Group C - possible human carcinogen and that for the purpose of risk characterization the MOE approach should be used for quantification of human risk.

This decision to classify pyrimethanil as a Group C carcinogen was based on evidence of increased incidences of thyroid tumors in both sexes of the Sprague-Dawley rat. Statistically significant increases were found for thyroid follicular cell adenomas and combined adenoma/carcinoma in male rats, and for thyroid follicular cell adenomas in female rats. The incidences of the thyroid tumors exceeded that of historical controls, in both sexes of the rat.

The CPRC agreed that the highest dose was not adequate for assessing the carcinogenic potential of pyrimethanil in the CD-1 mouse study; however, the CPRC agreed that the Registrant would not be asked to repeat the mouse study at this time.

Pyrimethanil is a new chemical for which no suitable analogs could be found and it does not appear to have mutagenic activity.

The CPRC also considered the possibility of using the threshold model for thyroid neoplasms based on the Agency's DRAFT Policy Document, "Thyroid Follicular Carcinogenesis: Mechanistic and Science Policy Considerations, SAB Review Draft, May 1988."

Applying the criteria in this draft policy, the CPRC concluded that there appeared to be sufficient evidence for relating the thyroid tumors in the rat to a disruption of the thyroid-pituitary status (a full discussion of this analysis is found in the body of the document - Section F, number 6).

Therefore, based on the evidence that pyrimethanil appears to induce thyroid tumors through a disruption in the thyroid-pituitary status, and thus may have a threshold for tumor development, the Committee recommended that a Margin of Exposure (M.O.E.) approach be used for quantitating carcinogenic risk, based on the precursor lesions in the rat thyroid and/or liver.

H. Induces Cancer Call -- Pyrimethanil

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to pyrimethanil resulted in an increased incidence of thyroid follicular cell tumors in both sexes of the Sprague-Dawley rat. Although the tumors were mainly benign, they had an early onset and follicular cell adenomas are known to progress to carcinoma. The evidence suggests that these tumors appear to be induced through a disruption in the thyroid-pituitary status.

The Committee agrees that pyrimethanil induces cancer in animals.